REVIEW

The force within: endocardial development, mechanotransduction and signalling during cardiac morphogenesis

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ABSTRACT

Endocardial cells are cardiac endothelial cells that line the interior of the heart tube. Historically, their contribution to cardiac development has mainly been considered from a morphological perspective. However, recent studies have begun to define novel instructive roles of the endocardium, as a sensor and signal transducer of biophysical forces induced by blood flow, and as an angiocrine signalling centre that is involved in myocardial cellular morphogenesis, regeneration and reprogramming. In this Review, we discuss how the endocardium develops, how endocardial-myocardial interactions influence the developing embryonic heart, and how the dysregulation of blood flow-responsive endocardial signalling can result in pathophysiological changes.

KEY WORDS: Endocardium, Cardiac development, Hemodynamics, Bmp, Krüppel-like factor 2, Vegf, Mechanotransduction, Zebrafish, Mouse

Introduction

During cardiac tube formation, cardiogenic progenitor cells give rise to endocardial cells that undergo a unique process of vasculoogenesis and assemble into a specialised endothelial bed that lines the interior of the heart and connects with the rest of the vascular network. Besides forming an integral part of the vasculature, these endocardial cells are crucial for the development of key cardiac structures (Fig. 1). For example, subpopulations of endocardial cells contribute to the formation of endocardial cushions and valve leaflets (Combs and Yutzey, 2009; Person et al., 2005) and to the interventricular and atrial septa (Snarr et al., 2008). Furthermore, signalling from endocardial cells is involved in the formation of trabeculae (Gassmann et al., 1995; Lee et al., 1994). After gastrulation, progenitor cells with endothelial and hematopoietic potential become positioned within the anterior lateral plate mesoderm (ALPM), which is located rostrally to a spatially separate population of heart and neural crest derivatives expressed 2 (hand2)-expressing myocardial progenitors. At this stage, endocardial progenitors express the endothelial marker genes tyrosine kinase with immunoglobulin-like and EGF-like domains 2 (tie2; also known as tek), T-cell acute lymphocytic leukemia 1 (tal1), and ets variant 2 (etv2; also known as etsrp) (Bussmann et al., 2007; Schoenebeck et al., 2007), supporting a common origin for endocardial and other endothelial cells. Lineage tracing of cells within the ALPM has shown that some cells contribute to the endocardium of the ventricular chamber (Schoenebeck et al., 2007). Similar studies in chick embryos showed that an endocardial progenitor cell population that is separate from myocardial progenitor cells arises at, or prior to, the primitive streak stage (Cohen-Gould and Mikawa, 1996; Lough and Sugi, 2000; Milgrom-Hoffman et al., 2011; Wei and Mikawa, 2000). These observations have contributed to a view (known as the ‘pre-specification’ model) that endocardial progenitors arise from a distinct lineage within the pre-cardiac mesoderm that is separate to that giving rise to myocardial cells.

By contrast, several lines of evidence suggest that myocardial and endocardial progenitors within the ALPM are related. In zebrafish cloche mutants, which lack endocardial cells (Stainier et al., 1995), or in embryos lacking the vascular and hematopoietic factors Tal1 and Etv2, the hand2 expression domain (which marks myocardial

Developmental origins of the endocardium

Lineage analyses in different vertebrate models have suggested that myocardial and endocardial progenitor cell populations have distinct developmental origins. In zebrafish, fate-mapping studies have shown that the entire myocardial and endocardial progenitor populations can be traced back to blastula stages (Keegan et al., 2004; Lee et al., 1994). After gastrulation, progenitor cells with endothelial and hematopoietic potential become positioned within the anterior lateral plate mesoderm (ALPM), which is located rostrally to a spatially separate population of heart and neural crest derivatives expressed 2 (hand2)-expressing myocardial progenitors. At this stage, endocardial progenitors express the endothelial marker genes tyrosine kinase with immunoglobulin-like and EGF-like domains 2 (tie2; also known as tek), T-cell acute lymphocytic leukemia 1 (tal1), and ets variant 2 (etv2; also known as etsrp) (Bussmann et al., 2007; Schoenebeck et al., 2007), supporting a common origin for endocardial and other endothelial cells. Lineage tracing of cells within the ALPM has shown that some cells contribute to the endocardium of the ventricular chamber (Schoenebeck et al., 2007). Similar studies in chick embryos showed that an endocardial progenitor cell population that is separate from myocardial progenitor cells arises at, or prior to, the primitive streak stage (Cohen-Gould and Mikawa, 1996; Lough and Sugi, 2000; Milgrom-Hoffman et al., 2011; Wei and Mikawa, 2000). These observations have contributed to a view (known as the ‘pre-specification’ model) that endocardial progenitors arise from a distinct lineage within the pre-cardiac mesoderm that is separate to that giving rise to myocardial cells.

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models may be to assume that cardiogenic progenitors remain multipotent throughout cardiac crest stages, but that positional information influences their fates (Harris and Black, 2010). However, further experimental data are needed to support this interpretation.

A related but largely unexplored issue is whether endocardial cells originate from within the secondary heart field, and if/how such cells might be related to other cardiac progenitors originating from this region. The secondary heart field contains different populations of cardiac progenitors that are initially positioned outside the heart but then migrate into it and contribute to its growth (Buckingham et al., 2005). However, it is unclear whether these distinct groups derive from multi-potential or from lineage-restricted progenitor cells. Several lineage-tracing studies in mice have suggested that, until the cardiac crescent stage, the endocardium develops from multipotent progenitors that also contribute to other cardiac cell lineages (Cai et al., 2003; Moretti et al., 2006; Verzi et al., 2005). However, live imaging in quail embryos revealed that a distinct population of endothelial cells that lie medial to the cardiac crescent migrates and enters the heart at the arterial pole, and contributes only to the endocardium (Milgrim-Hoffman et al., 2011), providing evidence for the presence of distinct populations of lineage-restricted progenitors within the secondary heart field. It was further shown that quail vascular endothelial cells transplanted into the cardiac region of chicken hosts contribute exclusively to endocardium, but not to myocardium, demonstrating that the endothelial lineage is specified prior to heart formation (Milgrim-Hoffman et al., 2011); this work also showed that the vascular endothelium from a relatively late developmental stage can still contribute to the endocardial lineage.

The link between the secondary heart field and the endocardium has also been explored in zebrafish embryos (Zhou et al., 2011), which exhibit a region defined by the expression of latent Tgfβ binding protein 3 (Ltbp3) that shares characteristics with the anterior secondary heart field described in mammals. It was shown that, as in higher vertebrates, this region contributes to endocardial cells but only within the outflow tract region of the heart; no contribution to endocardial cells within the main chambers was detected. It was further shown, using Cre/loxP-mediated lineage tracing of GATA binding protein 4+ (gata4+) and nks2.5+ cell populations, that zebrafish secondary heart field progenitors are specified within the ALPM and contribute to endocardial cells only within the outflow tract region of the heart (Guner-Ataman et al., 2013). In line with this, and in contrast to higher vertebrates in which secondary heart field-derived endocardial cells contribute to a large portion of the right ventricular endocardium (Cai et al., 2003; Milgrim-Hoffman et al., 2011; Moretti et al., 2006; Verzi et al., 2005), endocardial growth of the zebrafish main cardiac chambers is largely independent of a secondary heart field contribution (Lazic and Scott, 2011; Dietrich et al., 2014). These findings confirm earlier observations that the zebrafish chamber endocardium is highly proliferative (De Pater et al., 2009).

**Endocardial differentiation: interactions with myocardial progenitor cells**

There is evidence that endocardial and myocardial progenitors interact from the earliest stages of their development; indeed, the formation of an endocardial vascular bed and the development of the myocardium are closely connected. In zebrafish, endocardial progenitors start to express the endocardium-specific marker nuclear factor of activated T-cells, cytoplasmic, calcineurin-
dependent 1 (nfatc1) only after coming in close contact with myocardial progenitors at the embryonic midline (Wong et al., 2012). Accordingly, endocardial progenitors that fail to reach the embryonic midline lack nfatc1 expression (Wong et al., 2012), which in zebrafish is the first molecular indication that endocardial progenitors fail to undergo the radial movements seen in wild type and form dysmorphic cardiac cones. (B) Myocardial cell (MC) Tmem2 expression is involved in the migration of MC and endocardial cell (EC) progenitors. Correct levels of Fn1a are required for MC and EC migration, and Fn1a deposition is likely to be negatively regulated by myocardial Hand2. EC migration requires Tal1, which plays dual roles in the establishment of intercellular junctions and the suppression of myocardial cell fates. VEGF signalling is required within ECs for midline migration; whereas Slit2/Robo1 signals positively regulate this process, Slit2 exerts negative regulation on Vegfr2 through an unknown receptor. In addition, miR-218, which is encoded within slit2 introns, controls Robo1 receptor levels. A positive guidance cue for EC migration has not yet been identified. (C) The endoderm (blue) is essential for cardiac precursor migration. S1P signals from the yolk syncytial layer are sensed by the S1pr2 receptor, which activates Yap1 and is required for endodermal survival. S1P also activates Gα13/RhoGEF, which regulates convergence movements of the endoderm. This permits the migration of endocardial (green) and myocardial (red) cells. A transverse section through the cardiac field is shown beneath.

**Endocardial progenitor cell migration and cardiac tube assembly**

Upon specification, endocardial progenitors migrate into the heart field and contribute to the formation of a simple heart tube (Fig. 2A). Much of our understanding of this process has come from studies in zebrafish embryos. Despite their apparent molecular similarities with other endothelial cells, endocardial progenitors in zebrafish exhibit a migratory behaviour that is unique among all other endothelial progenitors. They migrate medially and posteriorly towards the midline (Fig. 2A), where they fuse in the region from which the heart cone will later arise (Bussmann et al., 2007). Studies have suggested that specified cardiac progenitors, including endocardial progenitors, acquire a competence to home into the cardiogenic region; when transplanted into zebrafish embryos, embryonic cells reprogrammed with Brg1 associated factor (BAF) chromatin remodelling complex components migrate and contribute strongly to different cardiac lineages including the endocardium, even when placed into ectopic transplantation sites outside the regions where cardiac progenitors are normally positioned during blastula stages (Lou et al., 2011).

Several factors and signalling pathways have been implicated in the midline-directed migration of endocardial cells in zebrafish (Fig. 2B). Migration towards the posterior is defective in mutants lacking the bHLH transcription factor Tal1. At later stages, endocardial progenitors cluster at the arterial pole and endocardial tissue elongation during cardiac tube formation is affected (Bussmann et al., 2007). These defects are connected to a breakdown of intercellular junction integrity in the endocardium (Schumacher et al., 2013). However, since Tal1 also functions as a repressor of myocardial identity, aberrant endocardial morphogenesis could be due to differentiation defects in tal1 mutants and further experiments are required to elucidate the precise mechanisms by which Tal1.

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Fig. 2. Endocardial-myocardial interactions during cardiac progenitor cell migration in zebrafish. (A) Scheme showing a dorsal view of endocardial (green) and myocardial (red) progenitor cell migration in wild-type (WT) and cloche mutant zebrafish embryos. The endocardium is required for myocardial radial movements. In cloche mutants, which lack endocardial cells, myocardial progenitors fail to undergo the radial movements seen in wild type and form dysmorphic cardiac cones. (B) Myocardial cell (MC) Tmem2 expression is involved in the migration of MC and endocardial cell (EC) progenitors. Correct levels of Fn1a are required for MC and EC migration, and Fn1a deposition is likely to be negatively regulated by myocardial Hand2. EC migration requires Tal1, which plays dual roles in the establishment of intercellular junctions and the suppression of myocardial cell fates. VEGF signalling is required within ECs for midline migration; whereas Slit2/Robo1 signals positively regulate this process, Slit2 exerts negative regulation on Vegfr2 through an unknown receptor. In addition, miR-218, which is encoded within slit2 introns, controls Robo1 receptor levels. A positive guidance cue for EC migration has not yet been identified. (C) The endoderm (blue) is essential for cardiac precursor migration. S1P signals from the yolk syncytial layer are sensed by the S1pr2 receptor, which activates Yap1 and is required for endodermal survival. S1P also activates Gα13/RhoGEF, which regulates convergence movements of the endoderm. This permits the migration of endocardial (green) and myocardial (red) cells. A transverse section through the cardiac field is shown beneath.
functions. Endocardial cells, similar to other endothelial cells, are also responsive to vascular endothelial growth factor (VEGF) signalling (Fig. 2B). The knockdown of vegfa or pharmacological inhibition of the VEGF receptor impairs heart formation at the zebrafish embryonic midline (Fish et al., 2011). Slit family ligands and their Roundabout (Robo) receptors regulate endocardial cell migration in a dosage-dependent manner, probably by modulating responsiveness to VEGF signalling; in this context, Slit/Robo signalling is controlled by miR-218, which is likely to target endocardial Robo receptors (Fish et al., 2011).

In zebrafish, the midline-directed migration of endocardial, as well as myocardial, progenitors depends on sphingosine-1-phosphate (S1P) signals from the yolk syncytial layer, and on the endoderm as a substrate for movement. In embryos mutant for S1P signalling components (Kawahara et al., 2009; Kupperman et al., 2000; Mendelson et al., 2015; Osborne et al., 2008) or in those with endodermal defects (Alexander et al., 1999; Kikuchi et al., 2000; Reiter et al., 1999) myocardial precursors fail to migrate to the midline. This causes celda bida – the formation of two lateral heart tubes that contain a central patch of endocardial cells (Holtzman et al., 2007; Osborne et al., 2008). Within endodermal cells, S1P signalling via the G-protein coupled receptor S1P receptor 2 (S1pr2) activates G-protein alpha 13a (Gna13a)/Rho guanine nucleotide exchange factor (RhoGEF)-dependent endodermal convergence movements (Ku and Lin, 2013; Ye et al., 2015) and ensures Yes-associated protein 1 (Yap1)-dependent endodermal survival (Fukui et al., 2014); this provides a permissive environment for both myocardial and endocardial cell migrations (Fig. 2C). Currently, there is no evidence for a role of S1P signalling during mouse endocardial progenitor migration, although this signalling pathway does play a role during the endodermal-to-mesenchymal transition (endMT) and migration of endocardial cells at the atrioventricular canal (Poulsen et al., 2011; Wendler and Rivkees, 2006).

The migration of early endocardial progenitors is also influenced by the myocardium. One candidate that mediates this is Transmembrane protein 2 (Tmem2), a single-pass transmembrane protein conserved in vertebrates that is expressed in both the myocardium and endocardium (Smith et al., 2011; Totong et al., 2011). tmem2 mRNA is contributed maternally and is required for the midline migration of cardiac progenitors; the correct fusion of myocardial and endocardial cell populations does not occur in maternal-zygotic tmem2 mutants (Totong et al., 2011). Surprisingly, the myocardial-specific expression of Tmem2 is sufficient to rescue both myocardial and endocardial midline migration defects in maternal-zygotic tmem2 mutants. Since the two tissues are not juxtaposed until they meet at the midline, a more indirect mechanism must therefore underlie the means by which myocardial Tmem2 guides endocardial midline migration (Fig. 2B). One proposal (Totong et al., 2011) is that myocardial Tmem2 provides a permissive environment for endocardial movements by modulating the extracellular matrix (ECM).

In addition to endocardial migration, correct heart tube formation requires the migration of myocardial cells (Fig. 2A), and this event is regulated by a variety of factors. In zebrafish, myocardial progenitors migrate as epithelial sheets and their integrity depends on deposition of the ECM component Fibronectin 1a (Fn1a) (Trinh and Stainier, 2004). Its loss causes defective adherens junctions, a loss of tissue integrity, a failure of cardiomyocytes to migrate towards the midline (Trinh and Stainier, 2004) and defective endocardial tube extension (Garavito-Aguilar et al., 2010). In zebrafish, the embryonic expression of Fn1a is positively regulated by the transcription factor Mix-type homeobox gene 1 (Mtx1; also known as Mtx1) (Sakaguchi et al., 2006) and dampened by the myocardial transcription factor Hand2 (Garavito-Aguilar et al., 2010). Accordingly, hand2 mutants have higher levels of Fn1a and this causes reduced anterior-posterior spreading of the early endocardium at cardiac cone stages (Garavito-Aguilar et al., 2010), an effect that is rescued when levels of Fn1a are reduced in hand2; fn1a double mutants. Since the production of Fn1a is affected by Hand2, which is expressed within myocardial but not endocardial progenitors (Schoenebeck et al., 2007), the myocardium apparently conditions an environment that is permissive for normal endocardial morphogenesis (Fig. 2B). Functional studies uncovered a similar requirement for Fibronectin during early endocardial morphogenesis in the mouse (Astrof et al., 2007; George et al., 1993, 1997). The phenotypes observed upon loss of Fibronectin include a collapse of the endocardium, endocardial cushion defects, or a severe lack of endocardial cells. Elucidating the precise mechanisms that contribute to a loss of endocardial cells will require additional experimental data.

High-resolution live imaging in zebrafish has revealed that myocardial progenitor cell migration towards the embryonic midline is also precisely choreographed and influenced by endocardial cells (Holtzman et al., 2007). By assessing myocardial cell movements in endocardium-deficient cloche mutants, Holtzman and colleagues showed that, although the initial coherent medial movement of myocardial cells towards the embryonic midline is not affected, later angular movements that result in the formation of a circular heart cone are disrupted (Fig. 2A). Angular movements of myocardial cells do not appear to be triggered by midline cues; they still occur in sphingosine-1-phosphate receptor 2 (s1pr2; miles apart) mutants in which myocardial cells fail to reach the midline (Chen et al., 1996; Kupperman et al., 2000). However, endocardial cells are required for the formation of the two lateral cardiac cones because in s1pr2; cloche double mutants, myocardial cells remain in coherent bilateral sheets without any angular movement. The precise molecular mechanisms by which endocardial cells guide myocardial movements during early cardiac morphogenesis, however, are currently unknown.

**Hemodynamic forces and cardiac morphogenesis: the role of the endocardium**

Following the assembly of the heart tube and the onset of blood flow, endothelial and endocardial cells are exposed to different hemodynamic forces, including frictional laminar shear stress, which is usually associated with healthy conditions, and turbulent wall shear stress, which is associated with pathological conditions (Hahn and Schwartz, 2009) and which may cause defective cardiac valve formation (Armstrong and Bischoff, 2004); both of these forces correlate with blood viscosity. In addition, endothelial and endocardial cells experience radial wall pressure due to hydrostatic pressure and this causes stretching of the cells. These biophysical forces change dynamically as the heart grows during development and as the strength and efficiency of the heart beat increases (Culver and Dickinson, 2010; Granados-Riveron and Brook, 2012). Thus, compared with other vascular beds, endocardial cells experience unique biophysical forces and forms of mechanical stress beyond fluid flow, including stretching during the diastole and contraction during the systole of each cardiac contractile cycle (Mickoleit et al., 2014). The molecular mechanisms by which endocardial cells sense these various hemodynamic forces and translate them into a response during cardiac development and homeostasis are currently the focus of intense investigations.
Mechanosensitive pathways within the endocardium

Blood vessel development and function are regulated to a large extent by hemodynamic forces (Boselli et al., 2015; Hahn and Schwartz, 2009). The endocardium is a specialised endothelium, and the mechanosensation and transduction mechanisms that it uses are thus likely to be similar to those used by other endothelial cells. Alterations to these hemodynamic forces, for example by surgical interventions in animal models, cause cardiac abnormalities that resemble human congenital heart defects (Midgett and Ruggonyi, 2014). Numerous important mechanosensory pathways have been identified in endothelial cells, but if and exactly how some of them function specifically in endocardial cells remains unclear.

One of the best-characterised mechanosensory pathways within endothelial cells involves platelet endothelial cell adhesion molecule 1 (Pecam1), VE-cadherin (Cadherin 5), Vegrfr2 (also known as Kdr) and Vegrfr3 (also known as Flt4) at cell-cell junctions (Baeyens et al., 2015; Conway and Schwartz, 2012; Conway et al., 2013; Coon et al., 2015; Tzima et al., 2005). Shear stress is transmitted from Pecam1 via VE-cadherin and results in Vegfr2/3 activation in a VEGF-independent manner. This triggers the production of phosphoinositides by PI3K that, in turn, activate integrins by recruiting several intracellular activators to their cytoplasmic tail (Collins et al., 2012). The precise role of this signalling pathway in endocardial development is still unknown, although it has been shown that the knockdown of VE-cadherin in zebrafish results in abnormalities in cardiac looping and defective endocardial cell junctions, along with increased endocardial permeability (Mitchell et al., 2010). However, this work did not address whether these defects resulted from changes in the ability of the cell to sense blood flow or from changes in endothelial cell adhesion. It is also becoming clear that not all of the mechanosensory pathways that are known to function in endocardial cell culture conditions are essential during cardiovascular development: unexpectedly, the murine knockout of Pecam1 causes only minor cardiovascular defects and mutant animals survive to adult stages (Chen and Tzima, 2009; Duncan et al., 1999; Schenkel et al., 2006). This finding indicates that we currently lack a complete understanding of the molecular mechanisms that regulate force-modulated endocardial development. Notably, it is possible that genetic redundancy or compensatory mechanisms may be masking the roles of some important components of mechanosensory pathways in vivo.

Other endothelial cell mechanosensory mechanisms involve primary cilia and ion channels, some of which may play similar roles in the endocardium. Primary cilia, which are utilised by endothelial cells to sense low shear forces during vascular morphogenesis (Goetz et al., 2014), are lost in the endothelium or endocardium over the course of zebrafish and chick development, presumably in response to higher shear stress (Egorova et al., 2011a; Goetz et al., 2014; Iomini et al., 2004). In chick embryos, they disappear from regions of high shear stress as the endocardium develops (at the cardiac cushions), but remain in regions where flow is low or disturbed (Egorova et al., 2011a). The loss of primary cilia at cardiac cushions is crucial for the Tgfβ/Alk5 (TgfBrs1)-dependent endMT of chick endocardial cells, which is induced by shear stress (Egorova et al., 2011a,b; Ten Dijke et al., 2012). Hence, it is possible that the bending and stability of cilia, which are differentially distributed within the cardiac tube in a flow-dependent manner, could generate sensory inputs that might explain why endocardial cells exhibit different responses to flow within different regions of the cardiac tube (Koeboed et al., 2014). Mechanosensitive ion channels have also been implicated in sensing oscillatory flows in the endocardium. For example, polycystic kidney disease 2 (Pkd2; also known as TRPP2) and transient receptor potential cation channel, subfamily V, member 4 (Trpv4) are implicated in sensing oscillatory flows in the atrioventricular canal region (Heckel et al., 2015). Piezo proteins, a class of recently identified mechanically activated ion channels, are also strong candidates for the mediation of flow responses in the endocardium (Coste et al., 2010, 2012; Kim et al., 2012). Piezo1, which is activated by shear stress in endothelial cells and is essential for vascular development in mice (Li et al., 2014; Ranade et al., 2014), is expressed in the mouse endocardium, and highest levels of Piezo1 expression are found in the atrioventricular canal and outflow tract (Ranade et al., 2014).

Different mechanosensory pathways activate flow-responsive genes within the endocardium. Currently, one of the best characterised of these genes encodes the zinc finger transcription factor Krüppel-like factor 2 (Klf2) (Novodvorsky and Chico, 2014). The human and murine KLF2/Klf2 genes possess a shear stress-responsive promotor element that is evolutionarily conserved (Huddleson et al., 2004, 2005) and are expressed in regions of high shear stress (Dekker et al., 2002; Groenendijk et al., 2004, 2005). Within the zebrafish atrioventricular canal, high oscillatory flow increases klf2a expression, which, as indicated by morpholino-mediated knockdown studies, is required for zebrafish valve development (Heckel et al., 2015; Vermot et al., 2009). Similarly, in mouse the loss of Klf2 causes defects in the endMT of endocardial cells at the atrioventricular cushions and atrial septation defects (Chiplunkar et al., 2013). On a cautionary note, however, it should be noted that zebrafish klf2a mutants do not exhibit any obvious cardiovascular defects (Novodvorsky et al., 2015). This could be due to genetic redundancy with klf2b, which is also expressed at cardiac cushions (Renz et al., 2015), or to some other genetic compensatory mechanism. Additional experiments are required to resolve this issue.

Klf2 expression is also controlled by the cerebral cavernous malformations (CCM) complex in mice and zebrafish (Renz et al., 2015; Zhou et al., 2015). CCM complex components play a role in controlling the response of β1 integrin signalling to blood flow in endothelial cells (Macek Jilkova et al., 2014), and the loss of CCM proteins in mouse and zebrafish results in severe cardiovascular defects (Boulday et al., 2009; Hogan et al., 2008; Klevaveland et al., 2009; Mably et al., 2003, 2006; Renz et al., 2015; Yoruk et al., 2012; Zheng et al., 2010; Zhou et al., 2015). Although Klf2 signalling has mainly been associated with vasoprotective functions within endothelial cells in response to shear stress (Dekker et al., 2006; Lee et al., 2006; Parmar et al., 2006), or with the control of cardiac valve morphogenesis in response to reversible flows (Heckel et al., 2015; Vermot et al., 2009), it has another important function: it promotes proangiogenic signalling during zebrafish aortic arch blood vessel development (Nicolì et al., 2010). Consequently, Klf2 upregulation in zebrafish CCM mutants results in increased VEGFR-dependent angiogenesis signalling and raises the proliferation rate of endocardial cells (Renz et al., 2015). This finding is in tune with earlier functional data showing that Ccm1 is an activator of Notch (Wüstehube et al., 2010), and that the loss of Ccm1 (also known as Krit1) or of its binding partner ICAP1 (also known as Itgb1bp1) results in Notch inhibition and an increase in angiogenesis signalling (Brütsch et al., 2010; Maddaluno et al., 2013; Wüstehube et al., 2010). The severe endocardial defects and loss of endocardial cushion formation in CCM mutants can be
explained by an earlier finding that the inhibition of Notch signalling causes increased Bmp6 expression; this, in turn, triggers pathological endMT (Maddaluno et al., 2013). It was recently shown that the loss of CCM proteins in mice activates the mitogen-activated protein kinase kinase kinase 3 (Map3k3; also known as Mekk3) signalling pathway, resulting in increased expression of ADAM metallopeptidase with thrombospondin type 1 motif 4/5 (Adams4/5) proteases, which degrade cardiac jelly, and increased levels of Klf2 (Zhou et al., 2015). Furthermore, it was found that the endocardial CCM mutant phenotypes in zebrafish are the result of the overexpression of Klf2a and Klf2b, which are activated by β1 integrin in a blood flow-independent manner (Renz et al., 2015). Taken together, these studies demonstrate that the CCM protein complex plays a crucial role in coupling mechanosensitive responses with downstream endocardial angiocrine signalling mediated by Klf2 (Fig. 3), although it is unclear if the CCM complex is regulated in a mechanosensitive manner.

The response to flow patterns is also mediated by small RNAs, often referred to as mechano-miRs (Kumar et al., 2014). In zebrafish, miR-143 is expressed within the outflow tract and ventricle in a flow-dependent manner, and its knockdown affects endocardial and myocardial cells and causes defects in ventricle and outflow tract formation (Miyasaka et al., 2011). miR-143 targets retinoic acid (RA) signalling pathway components (Fig. 3), which become ectopically expressed in response to miR-143 knockdown. This indicates that RA signalling activity is indirectly controlled by blood flow via miR-143 in the zebrafish heart. Within endothelial cells in culture, Klf2 binds to the promoter region of the miR143/145 cluster to induce expression (Hergenreider et al., 2012). Intriguingly, during cardiac chamber ballooning morphogenesis, miR-143 regulates the F-actin remodelling that is required for the elongation of ventricular cardiomyocytes. This process involves the repression of Adducin 3, an F-actin-capping protein (Deacon et al., 2010). In mouse, miR-92a controls the expression of Klf2 (Wu et al., 2011). Another small RNA that responds rapidly to blood flow is miR-21, which is expressed in regions of high shear stress in the zebrafish heart and suppresses a number of target genes that would otherwise impair valve formation (Banjo et al., 2013). Hence, flow-responsive proteins and small RNAs are required to transduce mechanosensation within the endocardium into a robust developmental output.

![Mechanosensitive pathways involved in endocardial signalling during ventricular chamber development, regeneration and reprogramming.](image)

Cilia sense fluid forces and induce endocardial Notch1 signalling, which controls the differentiation of adjacent myocardial cells into trabeculae. Neuregulin (Nrg) is secreted by endocardial cells (ECs) and activates ErbB2/ErbB4 receptors on myocardial cells (MCs) to induce trabeculation. EC Notch1 also positively regulates MC Bmp10, which is required to maintain MC proliferation, via a paracrine signal that has not yet been identified. EC Notch signalling is also required for MC reprogramming upon deletion of ventricular MCs by genetic means. RA signalling appears to be a permissive factor in an organ-wide injury response during regeneration. One candidate for the paracrine endocardial signal that stimulates cardiomyocytes to proliferate in response to RA or Notch signalling is Igf2b. RA signalling activity is indirectly controlled by blood flow via miR-143 in the zebrafish heart. During cardiac chamber ballooning morphogenesis, miR-143 also regulates the F-actin remodelling that is required for the elongation of ventricular cardiomyocytes, a process that involves the repression of Adducin 3. Klf2a mediates a transcriptional response that links the sensation of hemodynamic forces to the formation of distinct EC shapes and sizes. Klf2 also induces angiogenesis, and its expression is controlled by CCM complex proteins that suppress overactivation of β1 integrin and Mekk3 signalling, both of which, in turn, are strong inducers of Klf2. Mekk3 also induces Adamts4/5 proteases, which are involved in degrading cardiac jelly. Hence, the CCM protein complex couples mechanosensitive responses with downstream endocardial angiocrine signalling mediated by Klf2. Mekk3, Mek5 and Erk5 are also known as Map3k3, Map2k5 and Mapk7, respectively.
The effects of blood flow on endocardial and myocardial ballooning morphogenesis

During ballooning morphogenesis, the cardiac chambers grow into structures of distinct sizes, the inflow and outflow tract regions of the cardiac tube are brought into greater topological proximity by looping morphogenesis, and the boundary between the atrium and ventricle narrows at the atrioventricular canal, where the cardiac cushions will form (Fig. 4). During these events in zebrafish embryos, endocardial cells proliferate in a BMP-dependent and VEGF-independent manner and acquire distinct chamber- and region-specific morphologies (Dietrich et al., 2014). For example, within each cardiac chamber endocardial cells of the outer curvature become enlarged compared with those of the inner curvature. The reduction of shear stress via knockdown of the hematopoietic factors Gata1 and Gata2, which reduces the hematocrit (the abundance of red blood cells) and thus blood viscosity, causes an enlargement of endocardial cells within both heart chambers, indicating that shear stress restricts endocardial cell size. The flow-sensitive transcription factor Klf2a, which is induced within endocardium, has been identified as a key player in controlling endocardial cell shapes in zebrafish (Dietrich et al., 2014). Klf2a knockdown causes an expansion of endocardial cells and abolishes the differences in their sizes between the inner and outer curvature regions of the endocardium, while clonal overexpression of Klf2a reduces endocardial cell size. This suggests that Klf2a mediates a transcriptional response that links the sensation of hemodynamic forces to the formation of distinct endocardial cell shapes. A reduction in retrograde flow, which is the rate at which the blood oscillates between both chambers prior to the formation of cardiac cushions, also has a strong impact on endocardial cell proliferation (Dietrich et al., 2014).

Remarkably, myocardial cell morphogenesis corresponds to this blueprint established in the endocardium (Auman et al., 2007). In addition, genetic evidence in zebrafish has shown that a defective endocardium (e.g. in tal1 mutants) or a complete lack of endocardium (cloche mutants) causes myocardial ballooning defects (Bussmann et al., 2007; Holtzman et al., 2007; Schumacher et al., 2013; Stainier et al., 1995). Taken together,
these findings suggest that the endocardium functions as a sensor of blood flow and that this process induces changes within cells of the neighbouring myocardium in a manner that is essential for the morphogenetic adaptations of the cardiac tube in response to changing hemodynamic conditions.

Mechanosensitive endocardial signalling during cardiac cushion and valve formation

The morphogenesis of the embryonic cardiac cushions (from which cardiac valves are formed at later stages) involves an adaptation in endocardial tissue morphology (Fig. 4A,B) in response to changes in hemodynamics and to the contractile forces of neighbouring cardiomyocytes. With the formation of mature cardiac valves, hemodynamics in the heart change from retrograde flow (with oscillatory blood flow between the chambers) to a pattern of unidirectional flow. The genetic control of endocardial cushion and valve formation has already been the focus of a number of excellent reviews and involves a number of signalling pathways that coordinate the interaction between endocardial and myocardial cells (Armstrong and Bischoff, 2004; Kruthof et al., 2012; Singh and Kispert, 2010; Staudt and Stanier, 2012; Tian and Morrissey, 2012; Lagendijk et al., 2013), but here we focus on how biomechanical forces influence this process.

Work in zebrafish has been particularly important for understanding how the biomechanical force of blood flow impacts genetic pathways that control cardiac cushion formation because, unlike other vertebrate models, zebrafish embryos can survive for several days without a beating heart (Sehnert et al., 2002), thus unlike other vertebrate models, zebrafish embryos can survive for several days without a beating heart (Sehnert et al., 2002). With the formation of mature cardiac valves, hemodynamics in the heart change from retrograde flow (with oscillatory blood flow between the chambers) to a pattern of unidirectional flow. The genetic control of endocardial cushion and valve formation has already been the focus of a number of excellent reviews and involves a number of signalling pathways that coordinate the interaction between endocardial and myocardial cells (Armstrong and Bischoff, 2004; Kruthof et al., 2012; Singh and Kispert, 2010; Staudt and Stanier, 2012; Tian and Morrissey, 2012; Lagendijk et al., 2013), but here we focus on how biomechanical forces influence this process.

Work in zebrafish has been particularly important for understanding how the biomechanical force of blood flow impacts genetic pathways that control cardiac cushion formation because, unlike other vertebrate models, zebrafish embryos can survive for several days without a beating heart (Sehnert et al., 2002), thus making them accessible to manipulations that perturb blood flow. Zebrafish cardiac troponin T type 2a (tnnt2a) mutant embryos lack cardiac contractility and fail to form endocardial cushions (Bartman et al., 2004). Cardiac cushion development in zebrafish also occurs in a manner dependent on the biophysical force of blood flow (Auman et al., 2007; Vermot et al., 2009). Shear forces from retrograde blood flow are sensed by endocardial cells and drive cushion development until flows become unidirectional (Fig. 4A).

Following the formation of zebrafish embryonic cardiac cushions, an invagination of valve leaflets leads to the formation of cardiac valves (Beis et al., 2005; Scherz et al., 2008) (Fig. 4A,B) and intracardiac fluid flow is a critical regulator of this process (Hove et al., 2003). This morphogenetic process apparently differs from the maturation of cardiac cushions in mouse and humans, where the heart valve mesenchyme forms by an endMT of endocardial cells that delaminate and invade the cardiac jelly (Fig. 4B) (Chakraborty et al., 2010; Wu et al., 2013). In addition, genetic and pharmacological manipulations in zebrafish suggest that valve formation depends on retrograde flows rather than on the magnitude of shear stress to which cells are subjected (Vermot et al., 2009).

Currently, we lack a solid knowledge of how hemodynamics control cardiac cushion and valve formation in higher vertebrates due to the constant requirement of cardiac contractility and life-sustaining blood flow in these model organisms. In mice and humans, disturbed blood flow frequently manifests in cardiac cushion and valve defects (Midgett and Rugonyi, 2014). Hence, hemodynamic forces apparently induce mechanosensitive responses within endocardial cells that control morphogenetic changes in higher vertebrates (Culver and Dickinson, 2010). In addition, observations in chick embryos show that hemodynamic changes correlate with cardiac morphological changes (Yalcin et al., 2011). Furthermore, a recent report suggests that the formation of endocardial cushions in mouse is regulated by the mechanosensitive Hippo pathway (Zhang et al., 2014). Endothelial-specific conditional knockout of the transcriptional co-factor Yap1 results in hypocellular endocardial cushions; this phenotype is consistent with the well-established role of Yap1 in proliferation control and as a regulator of epithelial-to-mesenchymal transition in vitro. It is intriguing that the inactive form of Yap1 is prevented from entering the nucleus by interacting with the adherens junction cadherin-catenin complex, which itself is part of a mechanosensitive pathway.

The coupling of biochemical and mechanical signalling during cardiac valve morphogenesis is regulated by the mechanosensitive transcription factor Klfa2a in zebrafish (Heckel et al., 2015; Vermot et al., 2009) and Klf2 in mouse (Chiplunkar et al., 2013). Zebrafish klf2a morphants exhibit a large reduction in notch1b expression, which marks endocardial cushion differentiation, suggesting that retrograde blood flow acts through klf2a to promote endocardial cushion differentiation. Notably, klf2a morphants also display decreases in myocardial bmp4 expression, suggesting flow-responsive communication between the endocardium and myocardium during valve development (Vermot et al., 2009). As mentioned above, Kl2a is upregulated within the atrioventricular canal, and this requires endocardial Protein kinase D2 (Prkd2)-mediated suppression of nuclear Histone deacetylase 5 (Hdac5) activity, which acts as a negative regulator of klf2a expression (Just et al., 2011). The endocardium also responds to the mechanical stimuli from retrograde blood flows by activating the mechanosensitive Ca2+ channels Pkd2 and Trpv4, which transmit biomechanical forces exerted by oscillatory flow to regulate Klf2 expression (Heckel et al., 2015). In the absence of flow, Ca2+ levels and klf2a expression can be rescued using an agonistic peptide to activate Trpv4. It should also be noted that zebrafish embryos carrying loss-of-function alleles of pkd2 and trpv4 do develop valves but these display aberrant morphologies, suggesting that the activity of the two channels contributes to the control of valve morphogenesis rather than to the specification of endocardial cushions (Fig. 4C).

Viable zebrafish adult mutants with altered intracardiac flows have also provided insights into the way in which blood flow contributes to adult cardiac valve development (Kalogirou et al., 2014). For example, southpaw (nodal-related 3) mutants have a randomly positioned heart, and reverse flow fraction is increased in mutants that exhibit a midline-positioned heart; by contrast, it is decreased in myh6 mutants, which lack atrial contractility. Strikingly, the specification of endocardial cushion cells in both mutants appears almost normal at embryonic stages. However, these mutants exhibit defects in the remodelling of embryonic bicuspid valves, such that only two-cuspid or three-cuspid valves, rather than the four-cuspid valves normally found in adults, are formed (Kalogirou et al., 2014). Proper intracardiac flow dynamics therefore appear to be essential for the morphological remodelling that produces adult cardiac valves, in a manner that is independent of cardiomyocyte contractions (Kalogirou et al., 2014). A picture is thus emerging in which the initial specification of cardiac cushions is flow independent, but their further maturation and valve morphogenesis are sensitive to intracardiac flow patterns and depend on Klf2 function. This provides a fascinating example of a process whereby the sensing of immature flow patterns (the reverse flow fraction) triggers molecular pathways that control subsequent changes in valve morphology and ultimately ensure unidirectional blood flow.

ECM signalling in mechanotransduction during cardiac cushion formation

During cardiac cushion formation, the hydrated ECM between atrioventricular myocardial and endocardial cells plays a crucial
role as a sensor of biomechanical stress and as a mediator of intercellular communication between these two cell types. Biomechanical forces generated by the stiffness of the ECM also affect the development of endocardial cells. Extracellular proteins of the glycosaminoglycans, together with their associated sialic and hyaluronic acids, contribute to mechanotransduction pathways (Reitsma et al., 2007; Tarbell and Ebong, 2008). In line with this, endocardial cushion development is impaired in zebrafish and mammalian embryos with altered ECM compositions. For example, endocardial cushions are absent in hyaluronidase-treated rat embryos (Baldwin et al., 1994). Similarly, in mouse embryos deficient for hyaluronan synthase 2 (Has2), which controls the production of hyaluronic acid, endocardial cells fail to undergo endMT at cardiac cushions (Camenisch et al., 2000). In zebrafish, Has2 is regulated by miR-23, and is equally essential for endocardial cushion and valve formation (Lagendijk et al., 2011, 2013).

Studies in a chick in vitro model that uses a crosslinked hyaluronic acid hydrogel revealed that the mechanosensitive pathways that sense myocardial contractions are mediated by hyaluronic acid to induce an endMT of endocardial cells (Sewell-Loftin et al., 2014). Zebrafish jekyll mutants that lack uridine 5’-diphosphate (UDP)-glucose dehydrogenase, an enzyme essential for heparan sulphate, chondroitin sulphate, and hyaluronic acid production, also fail to form endocardial cushions (Walsh and Stainier, 2001). Given that proteoglycans regulate several signalling pathways – they affect the bioavailability and presentation of cytokines including BMPs and Wnts (Yan and Lin, 2009) – it is likely that they are important mediators of myocardial-endocardial communication and this might provide an alternative explanation for the endocardial cushion defects. Additional functional studies will be required to elucidate the precise molecular mechanisms involved.

The role of endocardial signalling and blood flow during trabeculation

During the course of ventricular morphogenesis, myocardial cells delaminate from the chamber wall and extend into the ventricular lumen to form a network of interconnected ridges – a process known as trabeculation (Moorman and Christoffels, 2003; Peshkovsky et al., 2011; Sedmera et al., 2000; Staudt et al., 2014). The formation of these myocardial ridges (trabeculae) is important for ventricular physiology; defects in their development can compromise cardiac function and cause cardiomyopathy (Oechslin and Jenni, 2011). The formation of trabeculae requires signalling from the endocardium to the myocardium, blood flow, and dynamic cellular movements.

The central signalling pathways involved in trabeculation include Neuregulin/Erb-b2 receptor tyrosine kinase 2 and 4 (ErbB2/ErbB4) (Gassmann et al., 1995; Lee et al., 1995; Liu et al., 2010; Meyer and Birchmeier, 1995; Morris et al., 1995; Peshkovsky et al., 2011; Tidcombe et al., 2003), Ephrin B2/EphB4 (Gerety et al., 1999; Wang et al., 1998), Semaphorin 6D/Plexin A1 (Toyofuku et al., 2004a,b), Notch1 (Grego-Bessa et al., 2007) and Bmp10 (Chen et al., 2004) signalling. Of note, work in mice supports a model in which endocardial Notch1 has two functions that act in parallel to control trabeculation. The first is the direct activation of endocardial Ephrin B2 expression, which promotes Neuregulin paracrine signalling to myocardial cells. The second function is to positively regulate myocardial Bmp10 to maintain myocardial proliferation through a paracrine signal that has not yet been identified but is known to act independently of Neuregulin (Grego-Bessa et al., 2007) (Fig. 3).

Trabeculation also requires intracardiac blood flow (Peshkovsky et al., 2011). In zebrafish cloche mutants, which lack endocardium, trabeculation is not initiated, suggesting that endocardial signals that are induced by flow instruct myocardial cells in this process (Peshkovsky et al., 2011). Similarly, zebrafish myh6 mutant embryos with reduced ventricular blood flow (Auman et al., 2007; Berdougo et al., 2003) fail to form trabeculae (Peshkovsky et al., 2011). It is plausible that the reduced blood flow in myh6 mutants might also have a negative effect on the myofibril maturation of ventricular myocardial cells (Lin et al., 2012); trabeculation defects could thus be a result of changes in sarcomere patterning that affect myocardial protrusions (Staudt et al., 2014). Some evidence suggests that Notch signalling may play a role in mechanosensitive pathways (Jahnsen et al., 2015). During zebrafish trabeculation, cilia play a role in sensing fluid forces and in inducing notch1b expression within the ventricular endocardium which, in turn, is required for correct trabeculation (Samsa et al., 2015). Studies in different model systems show that Notch expression defines regions within the embryonic endocardium that correspond with valve and chamber formation, and with ventricular trabeculation (De Luxán et al., 2015). However, it will be important to further elucidate the precise control of Notch signalling in the context of mechanosensitive processes including during trabeculation.

Dynamic cellular movements are required during trabeculation, and live imaging in zebrafish embryos has provided insight into this event (Staudt et al., 2014). This analysis shows that myocardial cells delaminate from the ventricular wall in a two-step process, first by extending membrane protrusions into the lumen, and subsequently moving their cell bodies through constrictions in the abluminal cell surface. The means by which the pattern of delaminating versus non-delaminating myocardial cells is determined remains unclear. Since endocardial Neuregulin is known to instruct myocardial cells to form trabeculae, it is possible that myocardial cells stochastically extend protrusions into the lumen, where they receive cues that determine their fates (Staudt et al., 2014). Shear forces sensed by the endocardium, as well as myocardial stretch forces, are also likely to affect pattern formation during trabeculation (Peshkovsky et al., 2011; Samsa et al., 2015). Thus, although the precise molecular and cellular mechanisms underlying trabeculation remain unclear, this process provides an excellent model for exploring the mechanisms underlying the interactions between endocardial and myocardial cells.

Signalling from the endocardium during cardiac regeneration and reprogramming

Endothelial cells serve as important angiocrine signalling centres in a plethora of processes, including during the growth and differentiation of tissues and organs and during tissue repair, and as stem cell niches during hematopoiesis (Ramasamy et al., 2015). It is therefore not surprising that the endocardium also contributes to cardiac repair and reprogramming. However, the molecular mechanisms by which it does so are poorly understood. In adult mammals, injuries to cardiac tissue usually cause irreversible damage and can lead to the formation of scars that affect cardiac function. By contrast, adult zebrafish cardiomyocytes exhibit a remarkable ability to proliferate and regenerate heart muscle even after substantial injuries (Gemberling et al., 2013; Kikuchi, 2014; Poss et al., 2002). Notably, non-muscle tissues – including the endocardium – actively help to drive the regeneration of the zebrafish myocardium.
The first and most obvious response to cardiac injury occurs within endocardium. Within hours after injury, endocardial cells throughout the entire heart switch to an ‘activated’ state, reflected in morphological changes such as cell rounding and detachment from the myocardium, and initiate strong expression of the genes encoding the RA synthesis enzyme Raldh2 (also known as Aldh1a2) and the transmembrane protein Heart of glass (Heg1), a component of the CCM protein complex (Kikuchi et al., 2011). While the role of Heart of glass during regeneration is unknown, it has been shown that RA signalling is part of an organ-wide injury response that becomes restricted to the site of the injury as regeneration proceeds. Indeed, RA signalling is required for myocardial proliferation, and inhibiting RA signalling in zebrafish impairs the proliferation of cardiomyocytes after injury (Kikuchi et al., 2011). The idea that RA signalling plays an important role in cardiomyocyte proliferation is consistent with studies of infarction in murine hearts, which lack regenerative capacity and do not trigger robust expression of Raldh2 in the endocardium or epicardium (Kikuchi et al., 2011). However, since treatment with exogenous RA or with a synthetic RA agonist does not affect cardiomyocyte proliferation in zebrafish, it is likely that RA signalling is permissive rather than instructive for heart regeneration.

The expression of Notch receptors is also upregulated within the adult zebrafish endocardium after myocardial injury, and Notch signalling positively regulates myocardial proliferation (Zhao et al., 2014). However, the nature of the paracrine endocardial signal that stimulates cardiomyocytes to proliferate in response to RA or Notch signalling is unknown. One candidate is Insulin growth factor 2b (Igf2b), which was discovered in a chemical screen for factors that affect cardiomyocyte proliferation in zebrafish (Choi et al., 2013). The expression of igf2b increases within the endocardium upon injury, and pharmacological manipulation of the insulin signalling pathway enhances or damps myocardial proliferation during heart regeneration (Choi et al., 2013). Identifying the key signalling factors via which the endocardium regulates myocardial proliferation during heart regeneration in zebrafish might prove helpful in therapeutic interventions in injured mammalian hearts.

An equally remarkable feature of the embryonic zebrafish heart is its capacity to compensate for the genetic ablation of the entire ventricular myocardium. Atrial cardiomyocytes respond to this massive loss of cells by transdifferentiating to ventricular fates and replenishing the ablated tissue (Zhang et al., 2013). Importantly, this transdifferentiation process requires endocardial Notch signalling, which is activated within the atrial chamber in response to ventricular ablation. Hence, a Notch-dependent cellular response within the endocardium is a common feature of zebrafish embryonic cardiomyocyte reprogramming and adult heart regeneration. Notably, Notch signalling activity is sensitive to blood flow within vascular endothelial cells (Watson et al., 2013) and endocardial cells of the atrioventricular canal (Vermot et al., 2009). This raises the intriguing possibility that altered hemodynamics caused by a loss of cardiomyocytes influences endocardial Notch signalling in the context of injury responses during heart regeneration.

Conclusions

Recent studies have challenged our view of the endocardium as a simple source of cells that contribute to cardiac structures. It is now appreciated as a key player that orchestrates cardiac morphogenesis, regeneration and reprogramming. This raises a number of questions for future research. For example, what molecular pathways does the endocardium use to regulate cardiac development and physiology? What is the basis of communication between the two cardiac tissue layers? Is this communication mediated by direct cell-cell contacts, extracellular vesicles, or factors that are secreted and directly traverse the ECM/cardiac jelly between the two layers of tissue? Attempts to find the answers to these questions must consider changes in the composition and organisation of the cardiac jelly in response to blood flow and cardiac contractility, as well as the way that adaptations of the ECM influence the availability and presentation of cytokines or the motility of extracellular vesicles.

Also largely unexplored are the mechanosensitive mechanisms underlying the responses of the endocardium to hemodynamic forces and cardiac contractility. Several mechanosensitive mechanisms with well-established roles in endothelial cells have not systematically been characterised within the endocardium. Until we have a reasonable inventory of the mechanosensitive mechanisms involved in these processes and the signalling pathways that lie downstream of them, it will be difficult to understand how endocardial cells integrate various hemodynamic forces to elicit distinct cellular responses (such as changes in cell morphology versus cell proliferation). Similarly, the relationship between mechanosensitive pathways and endocardial-myocardial communication remains unclear.

Further studies that aim to answer these questions and address these issues will no doubt provide insight into the processes that lead to congenital heart defects, which constitute the most common group of congenital defects in humans (Hoffman and Kaplan, 2002; Atkins andSucosky, 2014). They might also shed light on pathophysiological changes that develop in association with cardiac insufficiencies, which also affect the endocardium (Saffiz, 2011). Weakened cardiac pump functions reduce cardiac output, which would influence mechanosensitive response pathways within the endocardium. This might adversely feed back to the myocardium, initiating a vicious cycle that would enhance the initial effects of a cardiac insufficiency. Identifying the mechanisms that govern endocardial-myocardial interactions and the processes of mechanosensation that permit endocardial cells to sense the forces within the heart will be critical steps towards a better understanding of cardiac function and pathophysiology.

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Competing interests

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